ANTIDIABETIC EFFECTS OF A DIETARY SUPPLEMENT "PANCREAS TONIC"

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Pancreas Tonic, a dietary supplement, contains plant products shown to possess hypoglycemic activity. This study investigated the effect of Pancreas Tonic on serum glucose, glycosylated hemoglobin, and pancreatic islet cell regeneration of rats. Results showed that body weights of three groups of rats were not significantly different from each other before the study period, and after the 12 week study, weights increased with nonsignificant difference among the groups.

The diabetic group had significantly higher serum glucose levels compared with controls, and the diet-treated group had significantly lower serum glucose levels compared with the diabetic group. The diabetic group's glycosylated hemoglobin was significantly higher compared with the control group, and the diet-treated group had significantly lower glycosylated hemoglobin levels compared with the diabetic and control groups. Histological analysis of the pancreas showed a generalized reduction in size and number of islets in the diabetic group and regeneration of islet cells in the diet-treated group compared with the diabetic group. The diabetic group had a significant reduction in the number of cells compared with controls. The diet-treated group contained a significantly increased number of cells compared with the diabetic group. These data suggest that Pancreas Tonic induced an antidiabetic effect through pancreatic islet cell regeneration in experimental rats. (*J Natl Med Assoc.* 1998;90:614-618.)

Key words: diabetes ◆ dietary supplement ◆ Pancreas Tonic

Diabetes affects 1 in 20 Americans, with a disproportionately higher incidence among people of color and minorities. The investigation of new modalities for the prevention and control of diabetes is important because diabetes is a chronic disease. Particularly, nondrug and dietary approaches to diabetes are highly useful for minority populations, in whom access to health care is often a limiting factor.

Pancreas Tonic, a dietary supplement, is composed of several plant products and extracts of the Indian subcontinent. The majority of these ingredients either alone or in combination have been shown to have antidiabetic effects in diabetic animal models. For centuries, the Indian people have used these plant products in their diets as cooked or steamed vegetables, apparently deriving the benefits of regulating their blood sugar concentration, as the predominant ingredient in their diet is carbohydrate.

The extracts of seeds and leaves of the following plants are in Pancreas Tonic: *Cinnamomum tamale, Pterocarpus marsupeum, Momordica charantia, Azardichta indica, Tinospora cordifolia, Aegle marmelose, Gymnema sylvestre, Syzygium cumini, Trigonella foenum graecum,*

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and *Ficus racemosa*. The flavonoidal component epicatechin from *P marsupeum* has been shown to have antidiabetic action,[1] and more recent investigation of phenolic constituents demonstrated antihyperglycemic activity.[2]

Several types of extracts of *T foenum graceum* when administered orally to rabbits have been shown to have hypoglycemic activity.[3] Also known as fenugreek, *T foenum graecum* has shown antidiabetic activity in humans with diabetes[3] and in diabetic rats.[4] Another independent study with *T foenum graecum* demonstrated a dose-related hypoglycemic effect in alloxan-induced diabetic rats.[5]

Momordica chirantia, which is commonly known as "karela" in northern India, is consumed in the daily diet (in fruits). The hypoglycemic activity of its seeds was demonstrated in rats,[6] and its active principle was shown to possess antidiabetic activity.[7]

Gymnema sylvestre, another component of Pancreas Tonic, when administered orally to diabetic rats, lowered blood sugar level,[8] but later investigation in Japan[9] using *G sylvestre* did not show any improvement in insulin resistance in streptozotocin-induced diabetic rats. More recent independent studies from Japan[10] showed that some fractions extracted from *G sylvestre* inhibited the elevation of blood glucose by suppression of glucose uptake in the intestine.

To date, the information available pertaining to these plant products is either scanty or conflicting. This study investigated the effect of oral administration of Pancreas Tonic, which contains known or reported hypoglycemic plant products, on the biochemical and histological parameters of alloxan-induced diabetes in rats.

MATERIALS AND METHODS

Pancreas Tonic was prepared from the following plant products: *P marsupeum* (heartwood), 30%; *M charantia* (seeds), 6%; *G sylvestre* (leaves), 27%; *C tamale* (leaves), 3%; *A marmelose* (leaves), 6%; *A indica* (leaves), 3%; *T cordifolia* (stem), 5%; *T foenum graceum* (seeds), 10%; *F racemosa* (leaves), 2%; and *S cumini* (fruit), 8% (all percentages are expressed by dry weight). All of the plant products were crushed into coarse powder and soaked in water (1:8 W/V) for 24 hours. The soaked material was boiled with water until it was reduced to 25% of the initial volume and then filtered. The resultant extract was made into semisolid by low heat. The extract was air-dried at room temperature and made into powder, and mixed in the ratios described above.

Animals

Animals in this experiment were comprised of three groups of 10 Sprague-Dawley male rats (6 weeks of age). Animals were randomly assigned to one of the following groups:

- control group–rats in this group received no treatment and were placed on normal rat chow,
- diabetic group–rats in this group received an intraperitoneal injection of alloxan (80 mg/kg body weight) given after 4-6 hours of food withdrawal and placed on normal rat chow, and
- Pancreas Tonic group–rats in this group received an intraperitoneal injection of alloxan (80 mg/kg body weight) given after 4-6 hours of food withdrawal and placed on a normal diet for 5 days (to have alloxan-induced damage on pancreas) and shifted to Pancreas Tonic-supplemented (2% W/W) rat food.

Pancreas Tonic-supplemented food was prepared by Harlan Teklad (Madison, Wisconsin; diet formulation no. TD 96313). All of the animals were placed on these diets for 12 weeks. Body weights and feed consumption were recorded weekly. All animals were observed daily for general health and normal movements in the cages. No significant changes were observed in the overall health of these animals during the period of study.

Collecting and Processing of Blood Samples

Access to food was withdrawn for approximately 4 hours and animals then were anesthetized with ketamine-xylazine (80 mg-8 mg/kg body weight intraperitoneal injection). Blood samples were collected using a 10-mL syringe with an 18-gauge needle. Approximately 8-10 mL of blood was placed in a tube for serum and 3-4 mL was placed in a lavender top tube for glycosylated hemoglobin analysis.

Serum Biochemistry

All samples were coded with numbers, and the technician who performed the analysis was blinded to the group information. Serum samples were analyzed for chem-20 panel (serum glucose) using a standard autoanalyzer. Glycosylated hemoglobin concentration were determined using a routine gel electrophoretic method.

Preparation of Histological Slides

The pancreas was excised from the euthanized rats and fixed in 10% buffered formalin. Paraffin blocks were prepared, 5-μ sections were cut with a

Table 1. Body Weights of Control, Diabetic, and Pancreas Tonic-Treated Rats Before and After the 12-Week Study Period

Group	Initial Weight (g)	Final Weight (g)
Control	206±2.9	425±12.4
Diabetic	200±2.5	418±10
Pancreas Tonic	202±3.5	438±8.8

Table 2. Serum Glucose and Glycosylated Hemoglobin Concentrations in Control, Diabetic, and Pancreas Tonic-Treated Rats

Group	Serum Glucose (mg)	Glycosylated Hemoglobin (%)
Control	133±12.3	8.1±0.27
Diabetic	182±16.4 *	9.1±0.23 *
Pancreas Tonic	95±8.9*	7.0±0.29†

*Significantly higher compared with control group ($P<.01$).
†Significantly lower compared with diabetic group ($P<.0001$).

microtome, and routine microscopic slides were prepared. Hematoxylin-eosin staining was performed, and all slides were examined histologically for number of islets and total number of cells per pancreatic islet.

Statistical Analysis

All observations were first recorded in a note book, then entered into a Macintosh LCII computer and verified by another person for accuracy of data entry. Statistical analysis was performed using the StatView 4.5 (Abacus Concepts, California) software program. Each value contained at least 9 or 10 observations and were expressed as mean±SEM. The significance of differences among groups was determined by ANOVA and Fisher's PLSD test with probability values.

RESULTS

Body Weights

The initial (beginning of the study period) and final (end of study period) body weights of the control, diabetic, and Pancreas Tonic-treated groups are presented in Table 1.

Serum Glucose and Glycosylated Hemoglobin

Serum glucose concentration and glycosylated hemoglobin values are presented in Table 2.

Table 3. Number of Cells per Pancreatic Islet in Control, Diabetic, and Pancreas Tonic-Treated Rats

Group	No. Cells/Pancreatic Islet
Control	111±8.9
Diabetic	40±14*
Pancreas Tonic	79±8.8†

*Significantly lower compared with control group ($P<.02$).
†Significantly higher compared with diabetic group ($P<.02$).

Number of Cells per Islet

The histological examination of the pancreases was done, and the total numbers of cells per islet are presented in Table 3.

DISCUSSION

The data demonstrate that the overall body weights of the three groups of animals were similar (Table 1) at the beginning of the study and gradually increased through the study period with no significant differences among groups. This observation could mean that overall feed consumption and body metabolism were not altered by diet treatment. In addition, the daily feed consumption, general locomotion, and absence of any adverse symptoms among the diet group clearly suggest that Pancreas Tonic treatment did not produce adverse consequences in overall weight and health of the animals. This observation of no difference in body weights and normal feed consumption by the diet group suggests that mixing Pancreas Tonic in the rat feed did not detract from the taste of manufactured rat feed.

The serum glucose data suggest that intraperitoneal alloxan injections significantly elevated glucose concentration (Table 2), and the glycosylated hemoglobin levels support the fact the glucose elevations in serum were chronic in the diabetic group of rats. Pancreas Tonic treatment significantly decreased ($P<.0001$) the serum glucose concentration along with a significant reduction ($P<.0001$) in glycosylated hemoglobin in the diet-treated group. This is a significant finding, demonstrating not only the blood glucose lowering effect of Pancreas Tonic but also a reduction in glycosylated hemoglobin. Independent studies by other investigators[3,7] previously showed an antidiabetic effect of one or more

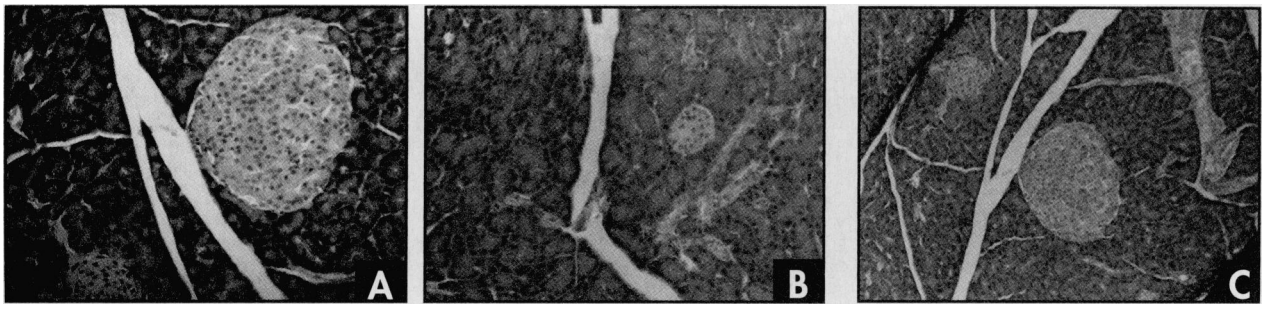

Figure 1.
Hematoxylin-eosin section showing morphology and number of cells in a pancreatic islet of control (A), diabetic (B), and Pancreas Tonic-treated (C) rats (original magnification 400 ×). The diabetic rat pancreatic islet shows a reduction in the size and number cells compared with the control rat pancreatic islet, while the Pancreas Tonic-treated rat pancreatic islet shows an increase in the size and number of cells.

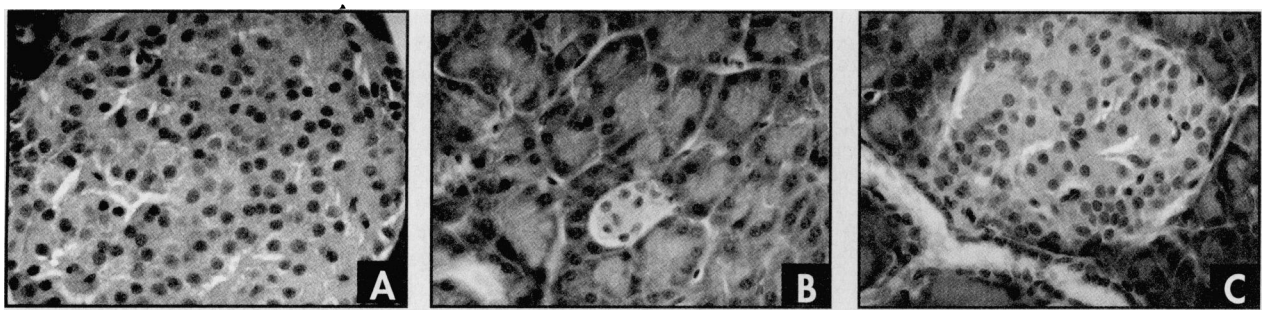

Figure 2.
Hematoxylin-eosin section showing morphology and number of cells in a pancreatic islet of control (A), diabetic (B), and Pancreas Tonic-treated (C) rats (original magnification 1000 ×). An increase in the number and size of pancreatic islet cells in the Pancreas Tonic-treated rat is apparent compared with those in the diabetic rat.

plant products that are components of the Pancreas Tonic, but our results present new evidence for a chronic reduction in blood glucose by Pancreas Tonic treatment.

The histological evidence provided in the present study (Figures 1-3 and Table 3) clearly demonstrate that alloxan injections destroyed the pancreatic ß-cells in the diabetic group of rats. A significant reduction in total number of cells per pancreatic islet was observed in the diabetic group, with a generalized shrinkage in size of islets. This observation supports the fact that an increase in serum glucose of diabetic rats was due to the damage done to pancreatic islets. The Pancreas Tonic-treatment group had a significantly higher number of cells per pancreatic islet, which suggests that Pancreas Tonic treatment regenerated the pancreatic islet cells; this complements the recent independent findings with *A* marmelose.[11] The histological observation of regeneration of islet cells in the Pancreas Tonic-treated group correlates with a significant reduction of serum glucose and glycosylated hemoglobin at the systemic level.

CONCLUSION

These findings suggest that Pancreas Tonic treatment induced a chronic reduction in serum glucose due to the regeneration of pancreatic islet cells. The underlying cellular and molecular mechanisms for these observed beneficial effects of Pancreas Tonic require further investigation.

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